Supplementary Information

Class I HDAC Inhibition Blocks Cocaine-Induced Plasticity Through Targeted Changes in Histone Methylation.

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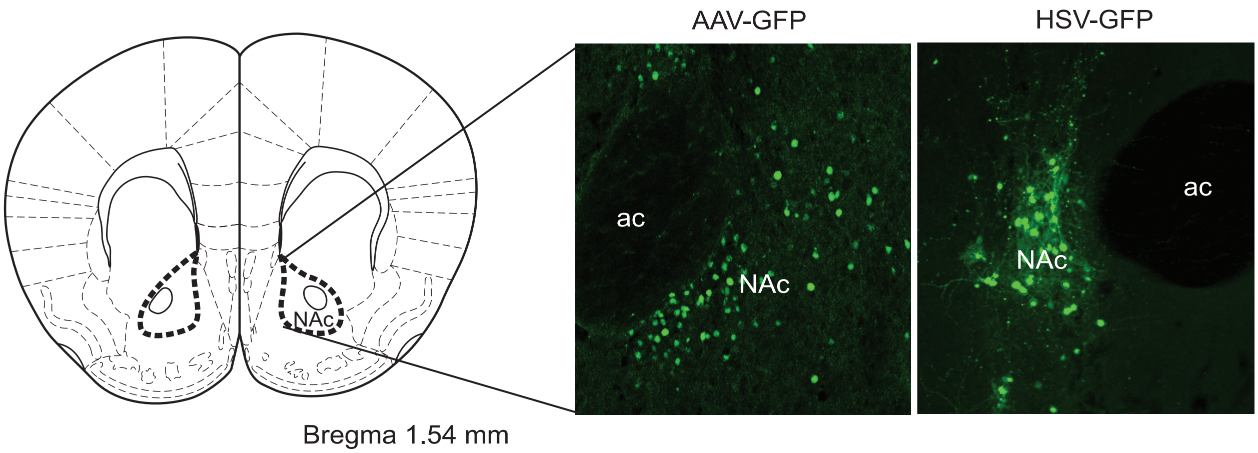
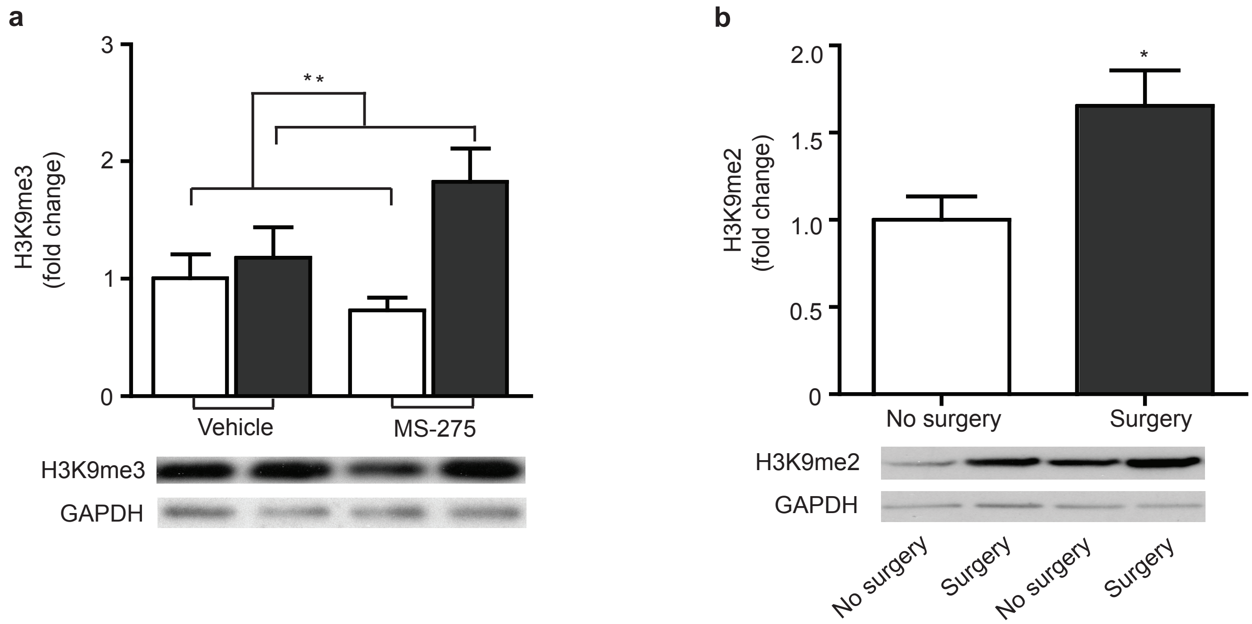
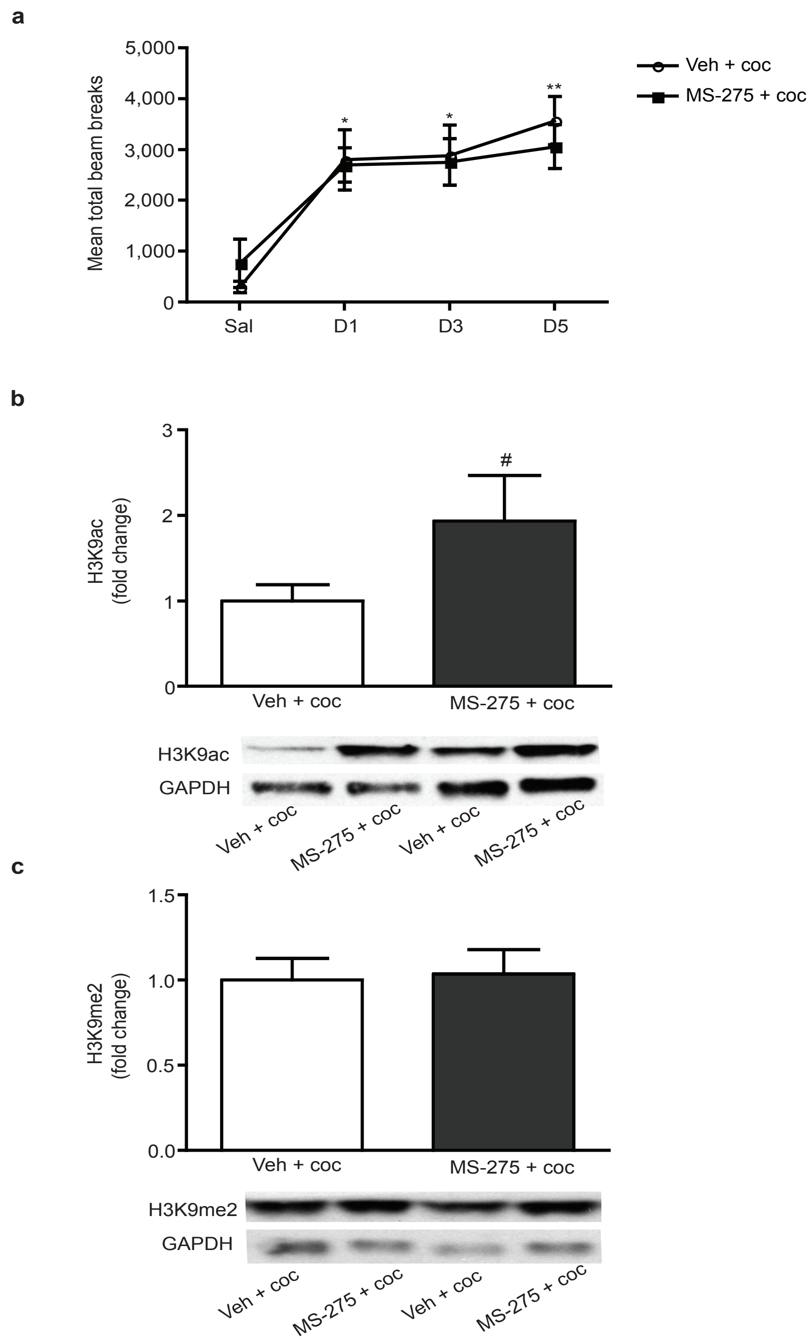


Supplementary figure 1. Representative image of AAV- and HSV-mediated transgene expression in the NAc.

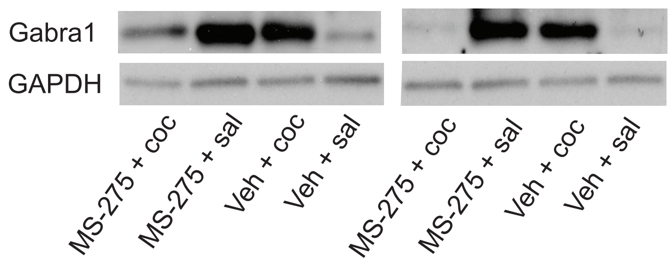


Supplementary figure 2. Repressive H3 histone methylation at lysine 9 is selectively altered in NAc by chronic MS-275 and cocaine treatments. (**a**) Chronic MS-275 infusion into the NAc increased levels of H3K9me3. A significant main effect (two-way ANOVA) of treatment ($F_{1,19} = 8.401$, $**P < 0.01$) was observed (N = 6 for vehicle + saline, vehicle + cocaine and MS-275 + saline treated groups, and N = 5 for MS-275 + cocaine group). (**b**) Minipump surgery plus vehicle infusion (i.e., in the absence of cocaine and MS-275) alone increased levels of H3K9me2 in the NAc (student's *t* test; $t_{14} = 2.663$, $*P = 0.0186$) (N = 8/group). (**c**) H3K4me3, and (**d**) H3K27me3 in NAc were unaffected by chronic MS-275 and cocaine treatments (two-way ANOVA; H3K4me3, no main effects of drug, $F_{1,9} = 0.1003$, treatment, $F_{1,9} = 0.005$, or interaction $F_{1,9} = 0.155$, $P > 0.05$; H3K27me3, no main effects of drug, $F_{1,9} = 0.219$, treatment, $F_{1,9} = 0.353$, or interaction $F_{1,9} = 0.008$, $P > 0.05$). All data are presented as mean ± s.e.m. Full-length blots are presented in Supplementary Figure 6.

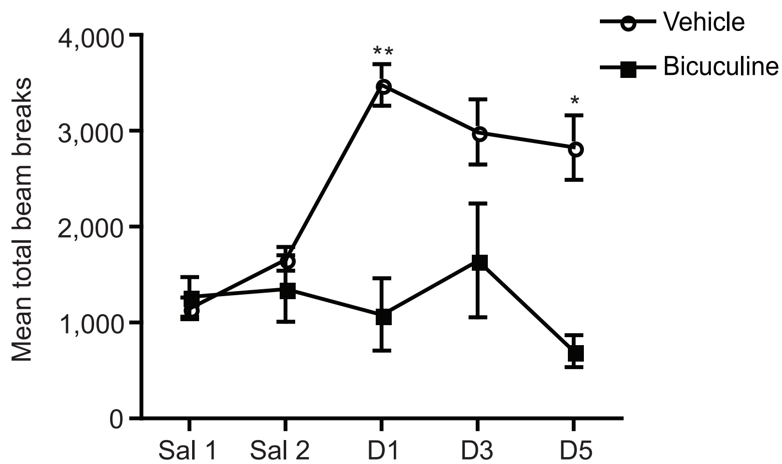


Supplementary figure 3. Acute MS-275 infusion into the NAc does not alter locomotor responses to cocaine and has no effect on repressive histone methylation. (**a**) Cocaine (10 mg/kg) locomotor activation in animals receiving acute daily intra-NAc infusions of vehicle (5 hydroxypropyl ßcyclodextrin) or MS-275 (100 µM). A significant (two-way ANOVA) effect of day ($F_{3,43}$ = 12.14 , P < 0.001) was observed . *P < 0.05 and **P < 0.01, Bonferroni *post hoc* tests (N = 7/group). (**b** and **c**) Global levels of H3 acetylation and methylation in the NAc 1 hour after 5 days of cocaine treatment paired with MS-275 (100 µM) (N = 7 or 9/group). All data represented as normalized values to GAPDH. (**b**) Acute MS-275 infusion into the NAc resulted in a strong trend toward an increase in levels of H3K9ac (planned student's t test; t_{12} = 1.633, # P = 0.0642, one-tailed). (**c**) Acute MS-275 infusion into the NAc did not alter levels of H3K9me2 (student's t test; (t_{16} = 0.1833, P = 0.8569). All data are presented as mean ± s.e.m. Full-length blots are presented in Supplementary Figure 6.

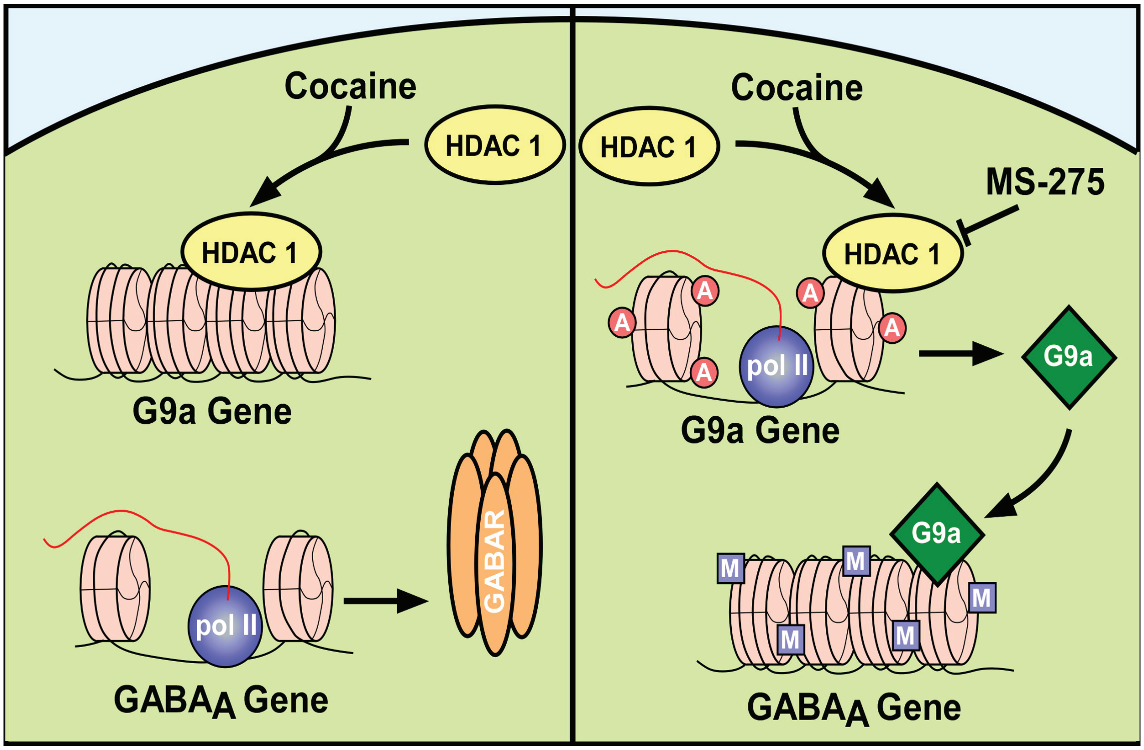
a



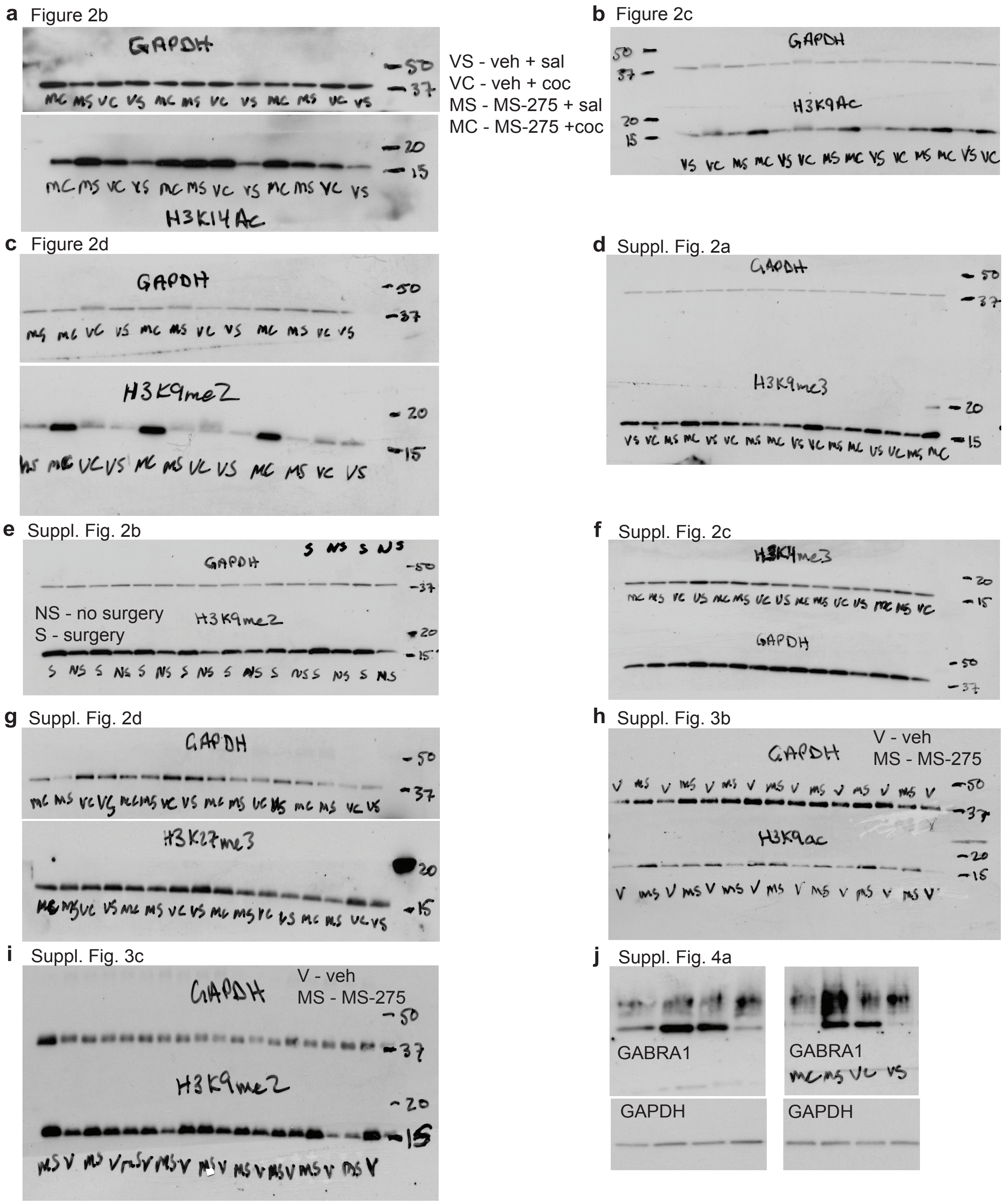
b



Supplementary figure 4. GABA$_A$ receptor function and cocaine/MS-275 action. (**a**) Qualitative western blot for GABRA1 in the NAc following 12 days of continuous treatment with MS-275 (100 µM) and 24hr after repeated cocaine (20mg/kg, seven daily doses). Two independent replicates are shown for each treatment group. Note the unique effect of combined MS-275 and cocaine treatment in producing no change in GABRA1 levels, unlike either treatment alone. Full-length blots are presented in Supplementary Figure 6. (**b**) Cocaine (10 mg/kg) locomotor activation in animals receiving acute daily intra-NAc infusions of vehicle (0.9% saline) or bicuculline (10ng), a competitive GABAA receptor antagonist. Bicuculline dramatically antagonizes locomotor responses to cocaine. A significant (two-way ANOVA) effect of day ($F_{4,40} = 3.571$, $P < 0.02$) and treatment ($F_{1,40} = 29.69$, $P < 0.001$) and an interaction between day and treatment ($F_{4,40} = 4.448$, $P < 0.01$) was observed. *$P < 0.05$ and **$P < 0.01$, Bonferroni *post hoc* tests (N = 6/group). Data are presented as mean ± s.e.m.



Supplementary figure 5. Schema depicting cocaine regulation of $GABA_A$ receptor subunit gene expression in the NAc through targeted chromatin modifications. Repeated cocaine targets HDAC1 to the *G9a*/*GLP* promoters, leading to decreased *G9a*/*GLP* gene expression and decreased binding of these KMTs at the promoters of certain $GABA_A$ receptor subunit genes. The resulting decreased repressive histone methylation (reduced H3K9me2) and loosening of chromatin at these promoters allows for increased transcription of the $GABA_A$ receptor subunits and increased inhibitory tone in the NAc. Chronic MS-275 treatment, by inhibiting HDAC1, promotes increased histone acetylation and increased *G9a*/*GLP* gene expression. These KMTs then catalyze increased H3K9me2 at $GABA_A$ receptor subunit gene promoters to block cocaine-induced transcriptional activation of the $GABA_A$ subunits and increased inhibitory tone.



Supplementary figure 6. Full-length western blots of representative images. Gels corresponding to figure 2 b-d (**a**, H3K14ac; **b**, H3K9ac; **c**, H3K9me2), supplementary figure 2a-d (**d**, H3K9me3; **e**, H3K9me2; **f**, H3K4me3; **g**, H3K27me3), supplementary figure 3b and c (**h**, H3K9ac; **i**, H3K9me2) and supplementary figure 4a (**j**, GABRA1).

Supplemental Table 1. Complete Primer List

Mouse mRNA primers

GAPDH-F	AGGTCGGTGTGAACGGATTTG
GAPDH-R	TGTAGACCATGTAGTTGAGGTCA
G9a-F	TGCCTATGTGGTCAGCTCAG
G9a-R	GGTTCTTGCAGCTTCTCCAG
GLP-F	ATTGACGCTCGGTTCTATGG
GLP-R	ACACTTGGAAGACCCACACC
Suv39H1-F	CTGTGCCGACTAGCCAAGC
Suv39H1-R	ATACCCACGCCACTTAACCAG
HDAC1-F	ATCAGCCCTTCCAACATGAC
HDAC1-R	TTGTCAGGGTCCTCCTCATC
HDAC2-F	CCCGAGGAGAACTACAGCAG
HDAC2-R	ACTCTTGGGGACACAGCATC
HDAC3-F	TCTGCCAAATGTTTTGGG
HDAC3-R	TCACAGATGGCTGTCAGG
HDAC5-F	TGTCACCGCCAGATGTTTTG
HDAC5-R	TGAGCAGAGCCGAGACACAG
HDAC7-F	GGTGGACCCCCTTTCAGAAG
HDAC7-R	TGGGTAGCCAGGAGTCTGGA
HDAC8-F	CATCGAAGGTTATGACTGTGTCC
HDAC8-R	GTTCTGGTGAAACAGGCTCTT
SIRT1-F	TTGGCACCGATCCTCGAAC
SIRT1-R	CCCAGCTCCAGTCAGAACTAT
GABRA1-F	GCAGATTGGATATTGGGAAGCA
GABRA1-R	GGTCCAGGCCCAAAGATAGTC
GABRA2-F	CCAGGACTGGGAGACAGTATT
GABRA2-R	CATTGTCATGTTATGGGCCACT
GABRA3-F	CACTAGAATCTTGGATCGGCTTT
GABRA3-R	CTTTCATCATGCCATGTCTGTCT

GABRA4-F	ACAATGAGACTCACCATAAGTGC
GABRA4-R	GGCCTTTGGTCCAGGTGTAG
GABRB1-F	TCTATGGACTACGGATCACAACC
GABRB1-R	ATTGACCCCAGTTACTGCTCC
GABRB2-F	AAACCGTATGATTCGATTGC
GABRB2-R	ACGATGGAGAACTGAGGAAGC
GABRB3-F	AAGACAGCCAAGGCCAAGAA
GABRB3-R	GCCTGCAACCTCATTCATTTC
GABRG1-F	ACTCAAGAAAATCGGATGCACA
GABRG1-R	ATGAAGTTGAAGGTAGCACTCTG
GABRG2-F	AGAAAAACCCTCTTCTTCGGATG
GABRG2-R	GTGGCATTGTTCATTTGAATGGT

Mouse promoter primers

G9a-F	GGGCAACCTGGGTAGGTAAT
G9a-R	AGCCCTCCTTGTGTCCTTTT
GLP-F	TGTTCCATCTTGGGAACACA
GLP-R	AGCCAGGGCTACACAGAGAA
Suv39H1-F	TGATGGAAACGGACATCTGA
Suv39H1-R	CCCAATTCTGCCAGTCATTT
GABRA1-F	GTTGGTAACTTGGGGCTTCA
GABRA1-R	GGGTCGATGCACTCTCAAAT
GABRA2-F	CTCCAGTGGGAGCCATACAT
GABRA2-R	TGTGTGTTTGTGAGGCCCTA
GABRA3-F	TGTTTTGCCTCCTTTGCTTT
GABRA3-R	GCTGTCAGCTCAGGCTTTCT
GABRB3-F	CAACCCCAGAAAGAGATGGA
GABRB3-R	GGCCTTGTCTTCCTCCCTAC
OCT4-F	CTGTAAGGACAGGCCGAGAG
OCT4-R	CAGGAGGCCTTCATTTTCAA